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Three-dimensional structure of recombinant human osteogenic protein 1: Structural paradigm for the transforming growth factor β superfamily

(x-ray crystallography/protein structure/bone morphogenetic protein/cystine knot/receptor binding model)

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ABSTRACT **We report the three-dimensional structure of osteogenic protein 1 (OP-1, also known as bone morphogenetic protein 7) to 2.8-Å resolution. OP-1 is a member of the transforming growth factor β (TGF-β) superfamily of proteins and is able to induce new bone formation *in vivo*. Members of this superfamily share sequence similarity in their C-terminal regions and are implicated in embryonic development and adult tissue repair. Our crystal structure makes possible the structural comparison between two members of the TGF-β superfamily. We find that although there is limited sequence identity between OP-1 and TGF-β2, they share a common polypeptide fold. These results establish a basis for proposing the OP-1/TGF-β2 fold as the primary structural motif for the TGF-β superfamily as a whole. Detailed comparison of the OP-1 and TGF-β2 structures has revealed striking differences that provide insights into how these growth factors interact with their receptors.**

Osteogenic protein 1 [OP-1, also called bone morphogenetic protein 7(BMP-7)] was originally isolated from bone based on its ability to induce new bone formation *in vivo* (1). Preclinical studies in nonhuman primate models have demonstrated that OP-1 is effective in restoring large segmental bone defects (2). Evidence that OP-1 is synthesized in the kidney (3) and is present in the circulation suggests that OP-1 has therapeutic potential for the treatment and management of osteoporosis and related metabolic bone diseases. In addition, tissue localization and other preclinical studies suggest that OP-1 has a role in the repair and regeneration of urogenital (4), neuronal (5), and cardiovascular (6) tissues. Knowledge of the three-dimensional structure of OP-1 is essential for understanding its mode of action and for providing a basis for the development of small molecule therapeutics.

OP-1 and transforming growth factor β2 (TGF-β2) are 2 of >30 homologous proteins in the TGF-β superfamily. Members of this superfamily have diverse biological activities and play critical roles in the migration, proliferation, and differentiation of mesenchymal cells during embryogenesis and in the repair and regeneration of tissues during postfetal life (7). These proteins are synthesized as large precursor proteins that undergo proteolytic processing at RXXR sites to yield mature active dimers of disulfide-linked monomers. Each monomer contains a conserved C-terminal 7-Cys domain with 20–92% sequence identity among superfamily members (7).

In this paper we report the three-dimensional structure of mature OP-1 to 2.8-Å resolution.¶ The overall OP-1 monomer fold is a Greek key motif with approximate dimensions 60 Å × 20 Å × 15 Å. In large part, the analogy to a left hand for TGF-β2 is true for OP-1 as well (8) (Figs. 1 and 2). The overall

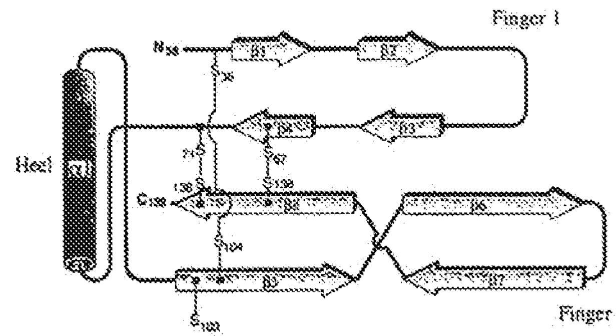

FIG. 1. Schematic drawing of the OP-1 monomer fold. The OP-1 cystine knot constitutes the core of the monomer and consists of three disulfide bonds; two, Cys-67–Cys-136 and Cys-71–Cys-138, form a ring through which the third, Cys-38–Cys-104, passes. The four strands of antiparallel β-sheet, which emanate from the knot, form two finger-like projections. An α-helix, located on the opposite end of the knot, lies perpendicular to the axis of the two fingers thereby forming the heel of the hand. The N terminus that corresponds to the thumb of the hand is unresolved in our electron density map. Unlike TGF-β2 (8, 9), this N-terminal region is not stabilized by a disulfide bond. The β-sheets are displayed as arrows and labeled from β1 through β8. The α-helix is displayed as a tube and labeled α1. Shown in solid thin lines are the intrasubunit disulfide bonds that make up the cystine knot. Starting from Gln-36, the residues involved in regular secondary structure are β1 (Lys-39 to His-41), β2 (Tyr-44 to Ser-46), β3 (Glu-60 to Ala-63), and β4 (Tyr-65 to Glu-70) in finger 1; β5 (Cys-103 to Asn-110), β6 (Ile-112 to Asp-118), β7 (Asn-122 to Tyr-128), and β8 (Val-132 to His-139) in finger 2; and α1 (Thr-82 to Ile-94) in the heel.

architectural similarity between OP-1 and TGF-β2 (8, 9) has allowed us to construct a structure-based sequence alignment (Fig. 3) from which we compared these two structures to begin to understand the chemical and structural elements involved in determining the specificity of these proteins to their receptors.

METHODS

Structure Determination. Crystals of human recombinant mature OP-1 were grown by mixing equal volumes of purified protein (10, 11), at 10 mg/ml, with 8% saturated ammonium sulfate in 50 mM sodium acetate (pH 5.0) (12). The crystals

Abbreviations: OP-1, osteogenic protein 1; BMP, bone morphogenetic protein; TGF-β, transforming growth factor β.
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¶The atomic coordinates and structure factors have been deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973 (reference BMP1). This information is embargoed for 1 year (coordinates) from the date of publication.

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